



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Analytical RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ramipril and Hydrochlorothiazide in Tablet Dosage Form

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ABSTRACT

A HPLC method for simultaneous estimation of Ramipril and Hydrochlorothiazide in tablets was developed and validated. The developed method involves Purosphere[®] Star Rp18e, 5 μ m, 150 \times 4.6mm column with mobile phase composition of acetonitrile and sodium perchlorate (pH 2.5) buffer in the ratio of 3:2, at a flow rate of 1.0 ml/min and UV detection at 316nm for first five minutes for Hydrochlorothiazide and 210nm for Ramipril. The method was validated as per ICH guidelines, Linearity was observed over concentration range of 17.5 to 32.5 μ g/ml for Ramipril and 87.5 to 162.5 μ g/ml for Hydrochlorothiazide. The Accuracy of the proposed method was determined by recovery studies and found to be 97.95-102.3% and 97.98-102.66% for Ramipril and Hydrochlorothiazide respectively. The proposed method was extended for estimation of Ramipril and Hydrochlorothiazide in marketed tablet formulation (Ramace-HTM) and it was found to be well within the acceptance limit. The developed and validated HPLC method for simultaneous estimation of Ramipril and Hydrochlorothiazide was found to be linear, accurate, precise, robust and rugged. Hence it can be used for routine analysis of Ramipril and Hydrochlorothiazide in tablets.

Keywords: HPLC, Ramipril, Hydrochlorothiazide, Ramace-HTM.

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Received 22 August 2014, Accepted 28 August 2014

Please cite this article in press as: Nagavi J *et al.*, Analytical RP-HPLC Method Development and Validation for The Simultaneous Estimation of Ramipril and Hydrochlorothiazide in Tablet Dosage Form. American Journal of PharmTech Research 2014.

INTRODUCTION

Market is flooded with combination of drugs in various dosage forms. Multicomponent formulations have gained lot of importance now a day due to greater patient acceptability, increased potency, multiple action, fewer side effects and quick relief. Simultaneous analysis procedures are now being used more frequently for estimation of drugs in multicomponent pharmaceutical formulations due to their inherent advantage viz., avoid time consuming, extraction and separation, economical in the sense that the use of expensive reagents is minimized and are equally accurate and precise. One such combination of drugs formulation is of Ramipril and Hydrochlorothiazide marketed as Ramace-HTM tablets, which is used in the treatment of hypertension. Ramipril^{1,2} is chemically (1S,5S,7S)-8-[(2S)-2-[[[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino]propanoyl]-8-azabicyclo[3.3.0]octane-7-carboxylic acid (Figure. 1A), is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. Hydrochlorothiazide, sometimes abbreviated HCT, HCTZ, or HZT is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide (Figure. 1B) is a popular diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water, used as antihypertensive^{1,3}. Ramace-HTM tablets contains Ramipril IP and Hydrochlorothiazide as API's, where Ramipril is a potent long acting ACE inhibitor and Hydrochlorothiazide is a thiazide diuretic and an antihypertensive. Ramipril blocks the counter regulatory rise in Angiotensin-II triggered by diuretic theory³. Few HPLC methods have been reported for estimation of Ramipril and Hydrochlorothiazide individually and in combination with other drugs. Since Ramipril and Hydrochlorothiazide is marketed in combination as tablets, there is a need to develop and validate a HPLC method for this combination of drugs. In the proposed project, an attempt shall be made to develop and validate a HPLC method and to apply the method for determination of Ramipril and Hydrochlorothiazide in tablets.

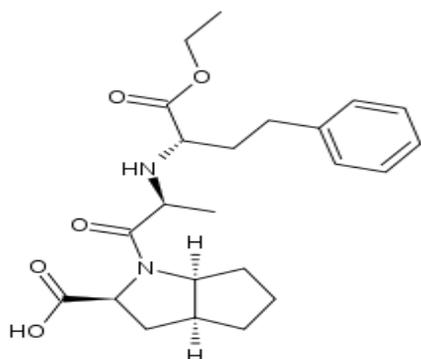


Figure 1(A):Ramipril

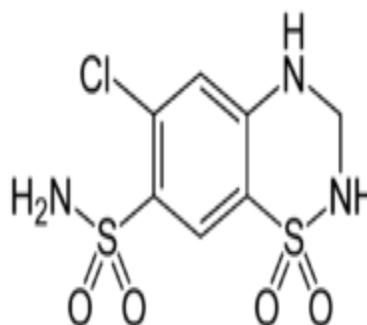


Figure 1(B):Hydrochlorothiazide

MATERIALS AND METHODS:

Chemicals & Reagents:

Water purified using Milli-Q, Acetonitrile HPLC, Orthophosphoric acid AR, Sodium perchlorate HPLC, Ramipril Working Reference Standard (WRS), Hydrochlorothiazide Working Reference Standard (WRS)

0.1M sodium perchlorate buffer:

Weigh accurately about 12.566 g of Sodium perchlorate in a beaker add 1000 ml of water and adjust the pH to 2.5 with 10% v/v Orthophosphoric acid.

Mobile phase:

To 650 ml of 0.1M sodium perchlorate buffer, add 350 ml of acetonitrile mix thoroughly filter through 0.45 μ membrane filter and sonicate for 2 minutes.

Diluents – mobile phase.

Ramipril standard stock (R₁). Weigh accurately and transfer about 12.5 mg of Ramipril (WRS), and transfer into a 25 ml volumetric flask. Add about 15ml of diluent, mix to dissolve. Dilute to volume with diluent and mix well, this is stock solution A.

Combined Standard Solution (CSS)

Weigh accurately about 12.5 mg of Hydrochlorothiazide in 100ml volumetric flask. Add about 30 ml of diluent and dissolve. Transfer volumetrically 5.0 ml of R₁ solution and dilute with diluent to volume and mix well, this is stock solution B.

Sample Preparation for Assay:

Note: Tablets should not be crushed or triturated in any way during the analysis.

Weigh accurately about 0.200g from above powder and transfer into a 100 ml volumetric flask. Add 40 ml of acetonitrile, sonicate for about 10 minutes. Cool to room temperature and make up to the mark using 0.1M sodium perchlorate buffer. Further dilute 5.0 ml of this solution to 100 ml with diluent and mix well. Filter the solution using 0.45 μ m syringe filter while injecting to HPLC.

Standard stock solution of Ramipril:

Weigh accurately and transfer about 12.5mg of Ramipril Working Reference Standard (WRS), and transfer into a 25ml volumetric flask. Add about 15ml of mobile phase, mix to dissolve. Dilute to volume with mobile phase and mix, to get the concentration of 500mcg/ml of Ramipril.

Standard stock solution of Hydrochlorothiazide:

Weigh accurately about 12.5mg of Hydrochlorothiazide WRS into 100ml volumetric flask and dissolve and make up the volume to 100ml with mobile phase, to get the final concentration of 125mcg/ml of Hydrochlorothiazide.

Preparation of mobile phase

To 650 ml of 0.1M Sodium Perchlorate buffer add 350 ml of Acetonitrile, mix filter through 0.45 μ m membrane filters and degassed by sonicating for 20min. A method was developed for determination of Ramipril and Hydrochlorothiazide on High performance liquid chromatograph (Agilent 1100 series), by selecting the optimum mobile phase, retention time, flow rate, λ_{max} based on resolution of Ramipril and Hydrochlorothiazide.

Instrument specifications:

Instrument	High performance liquid chromatograph Agilent 1100 series
Injector	Rheodyne.
Column	Purosphere [®] star Rp18e, 5 μ m, 150 \times 4.6mm
Detector	UV detector
Wavelength	316nm for first five minutes for Hydrochlorothiazide and 210 for Ramipril.
Flow rate	1.0 ml/min
Injection volume	10 μ l
Mobile phase	Acetonitrile: Sodium Perchlorate buffer (3:2)

Selection of mobile phase:

Ramipril and Hydrochlorothiazide are marketed as combined dosage formulation. The proposed method for estimation of Ramipril and Hydrochlorothiazide required adequate resolution of the two drug peaks in the chromatogram. Several solvent systems were tried to obtain good optimum resolution. Different solvents individually, in combination and in different ratios were tried. Selection of mobile phase was represented in Table 1.

Selection of Wavelength for detection:

The wavelength at which Ramipril and Hydrochlorothiazide showed maximum absorption was selected for our proposed method.

Procedure:

Ramipril 500 μ g/ml solution and Hydrochlorothiazide 125 μ g/ml solution were scanned individually on a UV–Visible spectrophotometer SHIMADZU 1700 in the wavelength range of 200 to 350 nm. The absorption spectrum of Ramipril and Hydrochlorothiazide obtained are presented in Fig 2 and 3.

Determination of Retention Time:

The retention time for Ramipril and Hydrochlorothiazide was determined by injecting 10 μ l of 500mcg/ml solution of Ramipril and 125mcg/ml of Hydrochlorothiazide at a flow rate of 1ml/min.

Determination of Retention Time for Ramipril:

Ramipril 500 μ g/ml solution was injected into the chromatograph with the flow rate of 1.0 ml/min. Peak area & retention time in chromatogram were observed, recorded and presented in Figure 4.

Determination of Retention Time for Hydrochlorothiazide:

Hydrochlorothiazide 125 µg/ml solution was injected into the chromatograph with the flow rate of 1.0 ml/min. Peak area and retention time in chromatogram were observed, recorded and are presented in Figure 5.

**Determination of Retention Time for Ramipril and Hydrochlorothiazide in combination:
Combined Standard Solution of Ramipril and Hydrochlorothiazide:**

Weigh accurately about 12.5mg of Hydrochlorothiazide WRS in 100ml volumetric flask; add about 50ml of mobile phase and dissolve. Pipette 5.0ml of standard solution of Ramipril into the same 100ml volumetric flask, dilute with mobile phase to volume and mix well (25mcg/ml of Ramipril, 125mcg/ml of Hydrochlorothiazide).

Procedure: 10 µl of the combined standard solution of Ramipril and Hydrochlorothiazide were injected and retention time, peak area and peak resolution were observed and the chromatogram is presented in Fig 6.

Determination of Optimum Flow Rate:

Various trials were carried out by altering the flow rates such as 1ml/min, 0.7ml/min, 0.5ml/min and 0.3ml/min to get good resolution of peaks of Ramipril and Hydrochlorothiazide. The flow rate which shows good resolution of Ramipril and Hydrochlorothiazide peaks was selected.

Determination of Resolution of Ramipril and Hydrochlorothiazide in combination.

A HPLC method was developed for simultaneous estimation of Ramipril and Hydrochlorothiazide using mobile phase of Acetonitrile and Sodium Perchlorate Buffer in the ratio of 3:2 at a flow rate of 1ml/min and UV detection at 316nm for first five minutes for Hydrochlorothiazide and 210nm for Ramipril.

Procedure:

10 µl of the combined standard solution of Ramipril and Hydrochlorothiazide was injected and retention time, peak area and peak resolution were observed and the chromatogram is presented in Figure 7 and Table 2.

VALIDATION OF DEVELOPED HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RAMIPRIL AND HYDROCHLOROTHIAZIDE

Since the HPLC method has been developed, validation of method by using various parameters was performed to ensure that the performance characteristic of the method meets the requirements for the intended analytical applications.

Following parameters were performed for method validation:

1. System suitability
2. Specificity
3. LOD (Limit of Detection)
4. LOQ (Limit of Quantification)
5. Linearity and Range
6. Precision
 - ❖ System precision
 - ❖ Method precision
 - ❖ Intermediate precision
7. Accuracy
8. Robustness
9. Stability of analytical solution

SYSTEM SUITABILITY:

These tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. This test ensures that the analytical system is working properly and can give accurate and precise results.

Procedure:

10 µl of Standard solution preparation of Ramipril and Hydrochlorothiazide was injected into chromatograph and chromatograms were recorded and presented in Table 3.

From the data obtained system suitability parameters like theoretical plates, tailing factor and resolution were calculated.

SPECIFICITY:

Specificity is the ability to assess unequivocally that the analyte in the presence of components which may be expected to be present; typically these might include impurities, degradation products and matrix components.

Procedure:

Inject 10 µl of blank solution in duplicate into the HPLC system and chromatograph.

Inject 10 µl of placebo solution (**P₁**) in duplicate into the HPLC system and chromatograph.

Inject 10 µl of Ramipril standard solution in duplicate into the HPLC system and chromatograph.

Inject 10 µl of Hydrochlorothiazide standard solution in duplicate into the HPLC system and chromatograph.

Inject 10 µl of Combined Standard Solution in duplicate into the HPLC system and chromatograph. Compare the chromatograms visually and check for any interference and Standard

chromatogram.

DETERMINATION OF DETECTION LIMIT FOR RAMIPRIL AND HYDROCHLOROTHIAZIDE (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Procedure:

10 µl of each solution of Ramipril and Hydrochlorothiazide were injected in to the chromatograph till the drug solution gives response but no peak area and chromatogram recorded. The concentrations of LOD for Ramipril and Hydrochlorothiazide are shown in Table 5.

DETERMINATION OF QUANTIFICATION LIMIT FOR RAMIPRIL AND HYDROCHLOROTHIAZIDE (LOQ):

Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Procedure:

10 µl of each solution of Ramipril and Hydrochlorothiazide were injected in to the chromatograph till the smallest concentration that gives response area and chromatogram recorded and shown in Table 5.

LINEARITY:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity level-1 solution: L₁ (70% of target concentration)

Dilute 7.0 ml of Standard Stock Solution (S₂) to 100ml with mobile phase.
(35, 87.5 mcg/ml of Ramipril and Hydrochlorothiazide respectively)

Linearity level-2 solution: L₂ (80% of target concentration)

Dilute 8.0 ml of Standard Stock Solution (S₂) to 100ml with mobile phase.
(40, 100 mcg/ml of Ramipril and Hydrochlorothiazide respectively)

Linearity level-3 solution: L₃ (100% of target concentration)

Dilute 10.0 ml of Standard Stock Solution (S₂) to 100ml with mobile phase.
(50, 125 mcg/ml of Ramipril and Hydrochlorothiazide respectively)

Linearity level-4 solution: L₄ (120% of target concentration)

Dilute 12.0 ml of Standard Stock Solution (S₂) to 100ml with mobile phase.
(60, 150 mcg/ml of Ramipril and Hydrochlorothiazide respectively)

Linearity level-5 solution: L₅ (130% of target concentration)

Dilute 15.0 ml of Standard Stock Solution (S₂) to 100ml with mobile phase.
(0.075, 1875 mcg/ml of Ramipril and Hydrochlorothiazide respectively)

Procedure:

Inject 10 µl of mobile phase into the HPLC system and chromatograph

Inject 10 µl of Linearity level-1 (L₁) solution six times

Inject 10 µl Linearity level solutions L₂, L₃, L₄, one time each, into the HPLC and chromatograph

Inject 10 µl of Linearity level-5 (L₅) solution six times and chromatograph.

The concentrations of Linearity for Ramipril and Hydrochlorothiazide are shown in Table 4.

The standard calibration graph is presented in Figure 8 and 9 for Ramipril and Hydrochlorothiazide respectively.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

(a) SYSTEM PRECISION

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area of six determinations is measured and % RSD should be calculated.

Procedure:

Successive six injections of 10 µl working sample solution were injected and chromatograms recorded. The % relative standard deviation was calculated for peak areas of replicates.

(b) METHOD PRECISION (Repeatability):

Method precision indicates whether a method is giving consistent results for a single batch, usually applied to standardization of methodology.

Procedure:

Successive six injections of 10 µl working sample solution were injected and chromatograms recorded. The % relative standard deviation was calculated for concentration of drug in replicates.

(c) INTERMEDIATE PRECISION (RUGGEDNESS):

Intermediate precision expresses within laboratories variations: (different days, different analysts, different equipment, etc.). The Intermediate precision parameter for Ramipril and Hydrochlorothiazide was performed. The method precision sets were repeated by different analyst, on different instrument using different lot of column, on different day. The % assay and %RSD for the same were calculated against standard solution for Method precision and Intermediate precision.

Procedure:

Successive six injections of 10 µl working sample solution were injected on different days and chromatograms recorded. The % relative standard deviation was calculated for concentration of drug in replicates. The standard deviation and Relative standard deviation were calculated from

statistical formula.

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amounts of analyte.

Recovery study for Ramipril and Hydrochlorothiazide at 70%, 100 and 130% levels:

Solutions for Accuracy study;

Accuracy level-1 solution: A₁ (70% of target concentration)

Weigh accurately about 0.200g weights of placebo and transfer to a 100 ml volumetric flask. Add 8 ml of stock solution (S₂) to the same volumetric flask, dilute up to the mark with mobile phase and mix. (17.5, 87.5 mcg/ml of Ramipril and Hydrochlorothiazide respectively). Prepare in triplicate.

Accuracy level-2 solution: A₂ (100% of target concentration)

Weigh accurately about one tablet weight of placebo and transfer to a 100 ml volumetric flask. Add 10 ml of stock solution (S₂) to the same volumetric flask, dilute up to the mark with mobile phase and mix. (25, 125 mcg/ml of Ramipril and Hydrochlorothiazide respectively). Prepare in triplicate.

Accuracy level-3 solution: A₃ (130% of target concentration)

Weigh accurately about one tablet weight of placebo and transfer to a 100 ml volumetric flask. Add 12 ml of stock solution (S₂) to the same volumetric flask, dilute up to the mark with mobile phase and mix. (32.5, 162.5 mcg/ml of Ramipril and Hydrochlorothiazide respectively). Prepare in triplicate.

Procedure

Inject 10 µl of mobile phase into the HPLC system and chromatograph the solution.

Inject 10 µl of combined standard solution (CSS) six times into the HPLC system and chromatograph the solution. Inject 10 µl of each of the accuracy solution A₁, A₂&A₃ individually, once into the HPLC and chromatograph the solution. Calculate the concentration in mcg/ml in the spiked placebo solutions by comparing with the standard solutions. Calculate % recovery. Report the average value for each level.

STABILITY IN ANALYTICAL SOLUTION:

The standard and the sample solutions need to be evaluated for the stability in the analytical solution by injecting at regular intervals. The stability in analytical solution for Ramipril and Hydrochlorothiazide standard solution was performed.

Procedure:

The standard solutions of Ramipril and Hydrochlorothiazide were injected at regular time intervals

for a period of 24 hours and the chromatograms were recorded to evaluate the stability of Ramipril and Hydrochlorothiazide in analytical solution.

RESULTS AND DISCUSSION:

Table 1: Selection of mobile phase for HPLC Method.

Mbbile phase used	Observation
Methanol (100%)	Only Ramipril detected
Water (100%)	Hydrochlorothiazide peak detected
Methanol: Sodium Perchlorate Buffer (7:3)	Only Ramipril detected
Methanol: Sodium Perchlorate Buffer (4:1)	Only Ramipril detected
Methanol: Sodium Perchlorate Buffer (2:1)	Only Ramipril detected
Methanol: Sodium Perchlorate Buffer (pH 6)	Only Ramipril detected
Methanol: Sodium Perchlorate Buffer (pH 4.5)	Only Ramipril detected
Methanol: Sodium Perchlorate Buffer (pH 3)	Only Ramipril detected
Acetonitrile (100%)	Only Ramipril detected
Acetonitrile: Sodium Perchlorate Buffer (3: 1)	Long retention time
Acetonitrile: Sodium Perchlorate Buffer (3: 2)	Long retention time
Methanol: Acetonitrile (3:1)	Only Ramipril detected
Methanol: Acetonitrile (3:2)	Only Ramipril detected
Acetonitrile: Sodium Perchlorate Buffer (3:2)	Satisfactory resolution

Selection of wavelength for detection:

Standard solutions of Ramipril (10 μ l/ml) and Hydrochlorothiazide (10 μ l/ml) were scanned in the wave length range of 200-400 nm using acetonitrile and sodium perchlorate buffer in the ratio of 3:2 as diluent and UV spectrum obtained is presented below.

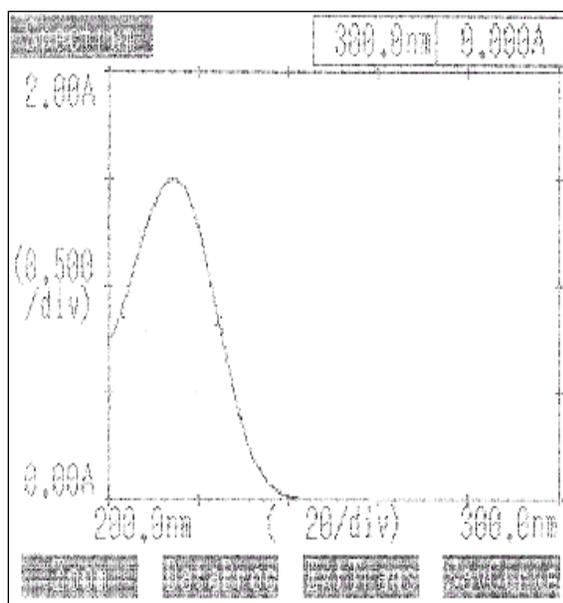


Figure 2: UV Spectrum of Ramipril

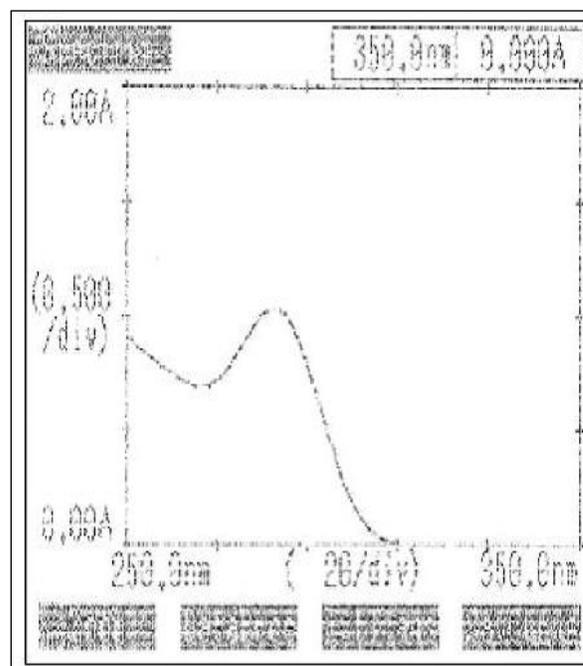


Figure 3: UV Spectrum of Hydrochlorothiazide

The 10 µg/ml solutions of Ramipril and Hydrochlorothiazide showed maximum absorbance at 210 nm and 316 nm was selected as wavelength maxima for determination of Ramipril and Hydrochlorothiazide.

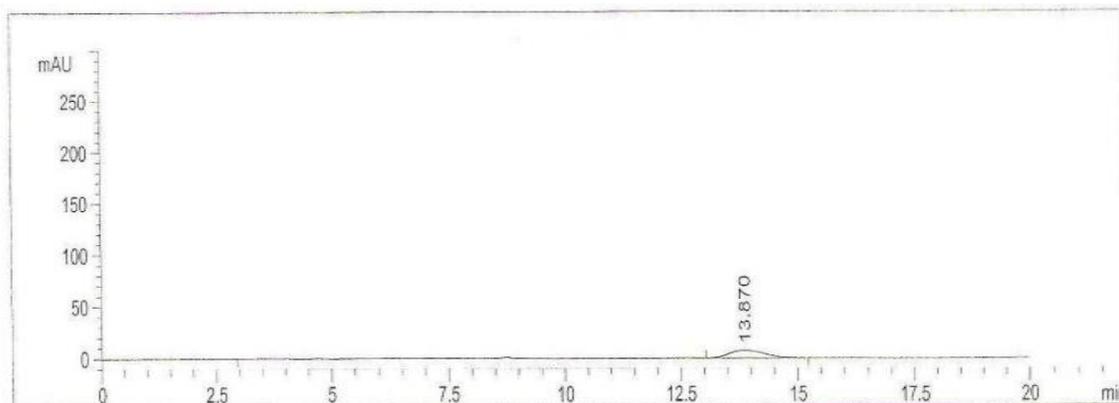


Figure 4: Chromatogram for determination of retention time for Ramipril.

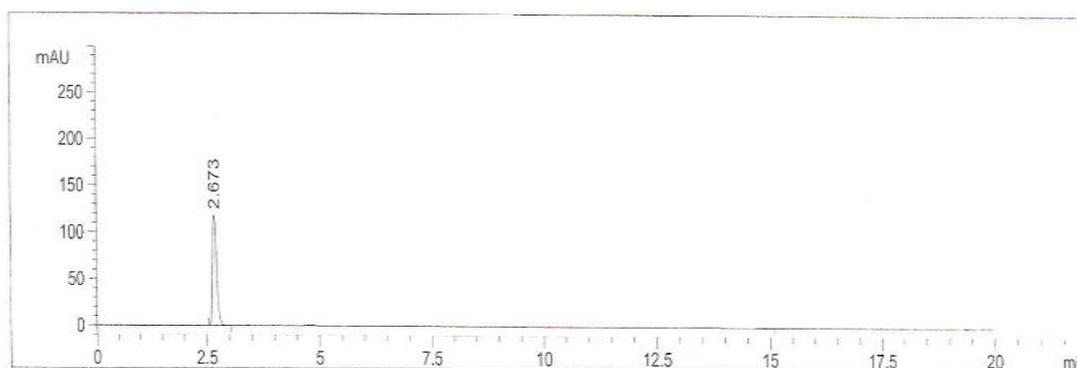


Figure 5: Chromatogram for determination of retention time for Hydrochlorothiazide

The retention time for Ramipril & Hydrochlorothiazide was found to be 13.87 mins & 2.673 mins.

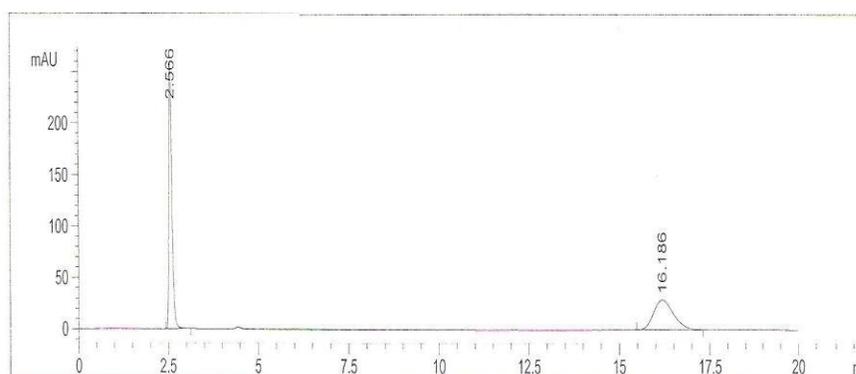


Figure 6: Chromatogram for simultaneous determination of retention time for Ramipril and Hydrochlorothiazide.

The retention time for Hydrochlorothiazide was found to be 2.566 mins and the retention time for Ramipril was found to be 16.186 mins.

Determination of Optimum Flow Rate:

The Optimum flow rate was found to be 1ml/min.

Table 2: Determination of Resolution for Ramipril and Hydrochlorothiazide in combination

Sl.No.	Ramipril		Hydrochlorothiazide	
	Peak area	Concentration	Peak area	Concentration
1	368.2	17.5	547.2	87.5
2	367.7	20	529.9	100
3	367.3	25	530.3	125
Average	367.7	20	535.8	100
SD	0.9	0.0142	0.9	0.0542
%RSD	0.2447	0.0712	0.1679	0.542
Resolution Factor			1.115	

Report:

The %RSD and the Resolution Factor obtained indicates that the method showed good resolution of Ramipril and Hydrochlorothiazide. It was found that the peaks were well resolved with retention time of 2.561 for Hydrochlorothiazide and 16.018 for Ramipril. Peaks of Ramipril and Hydrochlorothiazide were well resolved with the solvent system of Acetonitrile and Sodium Perchlorate Buffer in the ratio of 3:2.

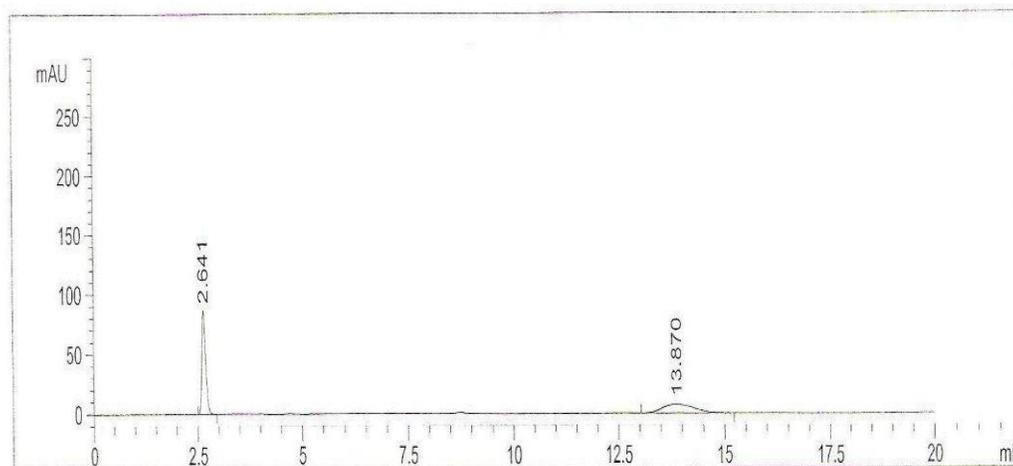


Figure 7: Chromatogram showing peak separation with Acetonitrile: Sodium perchlorate buffer (3:2) mobile phase.

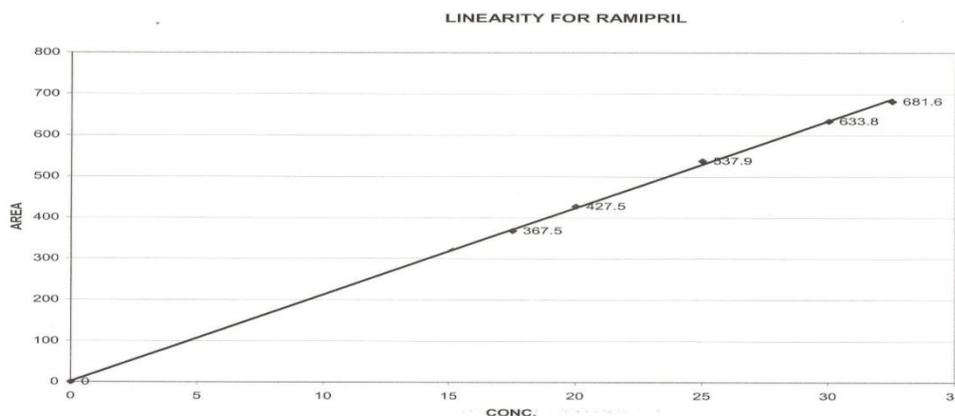
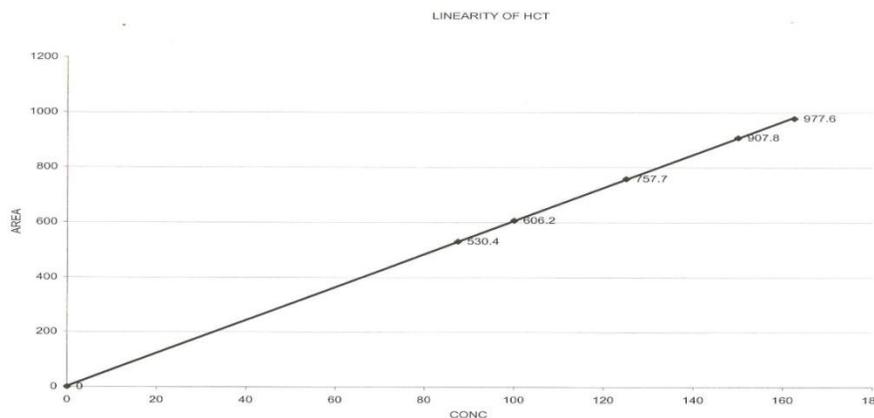
Table 3: Data for System Suitability parameters of Ramipril and Hydrochlorothiazide.

Parameters	Ramipril	Hydrochlorothiazide	Acceptance Criteria
Theoretical plates /meter	9772	46890	>2000
Theoretical plates /column	2443	11722	>2000
HETP	0.0102	0.0021	-
Symmetry factor	1	1	~1
Resolution	1.115		-

Table 4: Linearity Data for Ramipril & Hydrochlorothiazide

Sl. No	Ramipril Concentration (mcg/ml)	Peak Area *	Hydrochlorothiazide Concentration (mcg/ml)	Peak Area *
1	17.5	367.5	87.5	530.4
2	20.0	427.5	100.0	606.2
3	25.0	537.9	125.0	757.7
4	30.0	633.8	150.0	907.8
5	32.5	681.6	162.5	977.6

* Average of 3 values

**Figure 8: Standard calibration curve for Ramipril****Figure 9: Standard calibration curve for Hydrochlorothiazide****Table 5: Table for Stability of analytical solution**

Time interval	Ramipril		Hydrochlorothiazide	
	Peak Area *	Assay (%)	Peak Area *	Assay (%)
0 min	2813439	102.2653	4098658	99.37593
30 mins	2796564	101.6691	4026587	97.66596
60 mins	2793265	101.5525	4012359	97.32839
3 hours	2775624	100.9291	3982369	96.61684
6 hours	2756329	100.2473	3652489	88.79006
8 hours	2256487	82.58533	3216879	78.45472

*Average of 3 Readings

Report:

From the above data it was found that the Ramipril solution was stable for 6 hours and Hydrochlorothiazide solution was stable for 3 hours.

CONCLUSION:

An efficient high performance liquid chromatographic method was developed and validated for the simultaneous estimation of Ramipril and Hydrochlorothiazide. The HPLC method was developed using Agilent 1100 series C18, 150mm X 4.6mm, 5 μ column, and UV detection at 316nm for first five minutes for Hydrochlorothiazide and 210 for Ramipril., at a flow rate of 1.0 ml/min, and mobile phase of sodium perchlorate buffer and Acetonitrile in the ratio 3:2. The method was validated by using various validation parameters like accuracy, precision, linearity, specificity, stability in analytical solution and robustness. All the validation parameters were found to be well within the acceptance criteria. The HPLC method was found to be linear, accurate, precise, rugged and robust. Hence the method can also be applied for routine estimation of Ramipril and Hydrochlorothiazide in Ramace-H tablets. This work can be further extended to study the applicability of this method to determine Ramipril and Hydrochlorothiazide in biological fluids. Summary of validation parameters data for Ramipril and hydrochlorothiazide is presented in table 6.

Table 6: Summary of validation parameters data for Ramipril and hydrochlorothiazide

Parameters		Ramipril	Hydrochlorothiazide	Acceptance criteria
Retention Time (min)		15.28	2.75	-
LOD (ng/ml)		100	40	-
LOQ (ng/ml)		200	50	-
Linearity (μg/ml)		17.5-32.5	87.5-162.5	-
Accuracy (% Recovery)		97.95-102.3%	97.98-104.66%	97 -103%
Precision (%RSD)	System	1.78%	1.47%	< 2%
	Method	1.09%	0.77%	
	Intermediate precision	1.9819%	1.5619%	
Specificity		No peak of diluent, excipients and impurities were detected.		No peak should be detected
Robustness:	9:1	0.9551	0.2914	% RSD should be < 2%
Mobile phase ratio	7:3	1.0581	1.9081	
Robustness:	2.3	1.4709	1.1684	
pH of Sodium Perchlorate Buffer	2.7	1.8693	0.9177	
System Suitability	N	9772	46890	>2000
	HETP	0.0021	0.0102	-

Parameters	Asymmetry	1	1	~1
	Resolution	1.115		-

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the Principals, JSS College of Pharmacy and JSS Medical College, JSS University, Mysore for providing the necessary facilities to carry out the research work.

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